

中国横断山的地衣研究 (6): 灰梅属地衣修订*

刘 栋^{1,2}, 王欣宇², 李建文^{1,2}, 钱子刚¹, 王立松^{2**}(1 云南中医学院, 云南 昆明 650500; 2 中国科学院昆明植物研究所生物多样性
与生物地理学重点实验室, 云南 昆明 650201)

摘要: 通过对灰梅属 (*Canoparmelia*) 表型与基因型相结合的研究, 澄清了云南南部报道的珊瑚大叶梅 (*Parmotrema conformatum*) 是裂芽灰梅 (*C. ecaperata*) 的错误鉴定。由于珊瑚大叶梅和裂芽灰梅在经典分类鉴别上容易混淆, 通过 rDNA ITS 序列的系统发育分析, 证明了裂芽灰梅与粉芽灰梅 (*C. texana*) 及同心灰梅 (*C. conrescens*) 有较近的亲缘关系; 首次记录了粉芽灰梅在云南的分布范围; 文中对两个新记录种进行了详细描述和讨论, 并给出了中国已知灰梅属的检索表。

关键词: 灰梅属; 新记录; 地理分布; 分子系统学

中图分类号: Q 949. 34

文献标识码: A

文章编号: 2095-0845(2014)06-781-07

Contributions to the Lichen Flora of the Hengduan Mountains,
China (6): Revisional Study of the Genus *Canoparmelia*
(Lichenized Ascomycota, Parmeliaceae)*LIU Dong^{1,2}, WANG Xin-Yu², LI Jian-Wen^{1,2}, QIAN Zi-Gang¹, WANG Li-Song^{2**}(1 Yunnan University of Traditional Chinese Medicine, Kunming 650500, China; 2 Key Laboratory of Biodiversity and
Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China)

Abstract: A revision of *Canoparmelia* from the Hengduan Mountains (Yunnan, China) revealed the occurrence of *C. ecaperata*, based on specimens previously identified as *Parmotrema conformatum*. *C. ecaperata* is hereby reported as new to the flora of China. The study also led to the identification of *C. texana* as a component of the flora of Yunnan. In order to make sure the systematic position of these species, a phylogenetic analysis of rDNA ITS sequence was also carried out in this paper, indicating that *C. ecaperata* is closely related to *C. texana* and *C. conrescens*. Detailed information and discussion of these two new records are provided, together with a key to all the known taxa of *Canoparmelia* recorded from China. Following our revision, *Parmotrema conformatum* should be excluded from the flora of China.

Key words: *Canoparmelia*; New record; Geographic distribution; Molecular phylogeny

Canoparmelia Elix & Hale (Parmeliaceae, Lecanorales, Ascomycota) comprises ca. 40 species (Hawksworth *et al.*, 1995; Thell *et al.*, 2012) and is typified by *C. texana* (Tuck.) Elix & Hale. It was

segregated by Elix and Hale from *Pseudoparmelia* on the basis of morphological, geographic, ecological, and chemical characters (Elix *et al.*, 1986). Four species of *Canoparmelia* were previously known from

* Funding: The National Natural Science Foundation of China (No. 31170023, Y3216111W1), Foundation of Key Laboratory, CAS (KLBB-201210) and Flora Lichen Sinicorum (KSCX2-EW-Z-9)

** Author for correspondence; E-mail: wanglisong@mail.kib.ac.cn

Received date: 2013-07-22, Accepted date: 2013-08-05

作者简介: 刘 栋 (1988-) 男, 硕士, 主要从事地衣分类学研究。E-mail: liudong@mail.kib.ac.cn

China: two from Taiwan (Wei, 1991; Lai, 2000), and three from mainland China (Jia *et al.*, 2008). As part of an inventory of the lichen flora of the Hengduan Mountains (Yunnan, China) several specimens of *Canoparmelia* were collected. These belong to *C. ecaperata* Elix & Hale, which is a new record to the flora of China, and to *C. texana*, which is new to Yunnan Province. All specimens of *Parmotrema conformatum*, previously reported from Yunnan were misidentified and belong to *C. ecaperata*.

1 Materials and Methods

The specimens were mainly collected from Yunnan Province, and were deposited in KUN (Herbarium of Kunming Institute of Botany). External morphological descriptions were based on air-dried materials, and observation under stereomicroscopy (Dongwon OSM-1). Chemical characteristics were examined by medullary color reaction, with the solution for the color reaction following the methods described by Yoshimura (1974): K (10% aqueous KOH solution), C (NaClO solution), KC (10% KOH followed by C solution), P (5% alcoholic P-phenylenediamine). Thin layer chromatography (TLC) of acetone extracts was also carried out to further characterize the chemical profile of the specimens, using solvent systems C and G (Culberson and Hör-Dur, 1970; Orange *et al.*, 2001) and *Lethariella claudonioides* (Nyl.) Krog as control sample.

DNA was extracted from freshly collected and frozen herbarium specimens grinded to a fine powder, using Axyprep Multisource Genomic DNA Miniprep Kit following the manufacturer's instructions. The nuclear ITS (rDNA repeat) was amplified using primers ITS1 and ITS4 (White, 1990; Gardes and Bruns, 1993) in 25 μL reaction volume composed of 2 μL DNA, 1 μL of each primer, 12.5 μL 2 \times Taq PCR Mastermix (Aidlab) (contain Taq DNA Polymerase: 0.1 unit $\cdot \mu\text{L}^{-1}$; MgCl_2 : 4 mmol $\cdot \text{L}^{-1}$; dNTPs: 0.4 mmol $\cdot \text{L}^{-1}$), 8.5 μL dH_2O . Amplifications were carried out in an automatic thermocycler (C 1000TM), with the following profile: initial denaturation at 94 °C

for 5 min, followed by 30 cycles, each of three steps (94 °C for 1 min, 56 °C for 1 min, 72 °C for 1.5 min), and a final extension at 72 °C for 7 min.

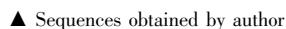
Twenty-seven sequences (18 retrieved from GenBank and 9 obtained by authors, Table 1) were assembled into a matrix for phylogenetic analyses. The sequence database was subjected to automated alignment and then manually inspected in BioEdit. *Hypotrachyna adducta* was set as the outgroup. Ambiguous regions were deleted. Bayesian inference of phylogeny was performed with in MrBayes v3. 1. 2. ModelTest 3.7 was used to estimate separate best-fit models of evolution for ITS. Bayesian analyses were carried out using the Metropolis-coupled Markov chain Monte Carlo method (MCMCMC). Analyses were run under the GTR model using a gamma-distributed rate parameter and a proportion of invariable sites. Two parallel MCMCMC runs were performed, each using four chains and 1 million generations, sampling trees of every 100th generation. The initial burn-in was set to 50%. A 50% majority-rule consensus cladogram was computed from the remaining trees; the proportions of this tree correspond to Bayesian posterior probabilities (BPP), and clades with $\text{PP} \geq 0.95$ were considered to be significantly supported. The phylogenetic trees are shown in Fig. 1.

2 Result

2.1 Key to the species of known *Canoparmelia* in China

1. Thallus without soredia or isidia *C. subtiliacea*
1. Thallus sorediate or isidiate
 2. Thallus sorediate
 3. Medulla P+ yellow, containing stictic and constictic acid acid *C. crozalsiana*
 3. Medulla P-, containing divaricatic acid *C. texana*
 2. Thallus isidiate
 4. Medulla P+ red, containing protocetraric acid *C. amazonica*
 4. Medulla P-, containing divaricatic and usnic acid *C. ecaperata*

Species name	Accession NO.	Location	Source	Species name	Accession NO.	Location	Source
<i>Canoparmelia concrescens</i>	GU994543	Kenya	GenBank	<i>F. papillosa</i>	HM010936	Uruguay	GenBank
<i>C. ecaperata</i>	KC978843	China	10-31934	<i>F. soredians</i>	AY586562	Spain	GenBank
<i>C. ecaperata</i>	KC978844	China	12-33440	<i>F. springtonensis</i>	EF042907	Australia	GenBank
<i>C. ecaperata</i>	KC978845	China	12-33611	<i>F. subambigua</i>	GU994551	Argentina	GenBank
<i>C. ecaperata</i>	KC978846	China	12-34474	<i>F. subambigua</i>	JN943839	Argentina	GenBank
<i>C. nairobiensis</i>	GU994545	Kenya	GenBank	<i>Hypotrachynia adducta</i>	KC978855	China	12-34288
<i>C. texana</i>	KC978847	China	12-37655	<i>Parmotrema subtinctorium</i>	KC978853	China	12-35202
<i>C. texana</i>	KC978848	China	12-37665	<i>P. norsticticatum</i>	GU994577	South Africa	GenBank
<i>C. texana</i>	GU994547	Kenya	GenBank	<i>P. reticulatum</i>	AY586579	Portugal	GenBank
<i>Flavoparmelia baltimorensis</i>	AY586559	USA	GenBank	<i>P. subcaperatum</i>	AY586557	Australia	GenBank
<i>F. caperata</i>	AY581059	Spain	GenBank	<i>P. subtinctorium</i>	GU593037	China	GenBank
<i>F. citrinescens</i>	GU994550	Argentina	GenBank	<i>P. tinctorum</i>	AY586570	Australia	GenBank
<i>F. haywardiana</i>	HM010933	New Zealand	GenBank	<i>P. tinctorun</i>	KC978854	China	12-33444
<i>F. marchantii</i>	JN943840	—	GenBank				



2.2 Taxonomy

Canoparmelia ecaperata Elix & Hale in Mycotaxon **27**: 277, 1986. ——— *Parmelia ecaperata* Müll. Arg. in Flora, Jena **74**: 378. 1891. ——— *Pseudoparmelia ecaperata* (Müll. Arg.) Hale in Phytologia **29**: 190, 1974.

Diagnostic characters: Thallus foliose, adnate on bark, grayish yellow to yellow-green, 4–14 cm in diam.; lobes irregularly sublinear, 1–3 mm wide, without cilia; upper surface plane, continuous, sometimes cracked on the old part; covered with dense isidia, simple and short, 0.1–1 mm long, cylindrical or clavate, mature ones with brown to black tips; medulla white, 62.5–75 μm thick; lower surface

black, turning brownish near the margin, rhizines rare, simple and black, 0.5–1.5 mm long; lower cortex 25–75 μm thick, Apothecia not seen (Fig. 2).

Chemistry: Cortex K-, medulla K-, C- (pink), KC-, P-; atranorin, divaricatic acid, usnic acid and an unknown compound (Rf value between classes 4 and 5).

Distribution: China: Yunnan Province, Nanjian Co. (Fig. 3); Africa, Nepal, India, Thailand (Hale and Mason, 1976; Singh and Sinha, 2010; Swinscow *et al.*, 1988; Wolseley *et al.*, 2002).

Habitat and ecology: This species grows on the bark of *Pinus yunnanensis* from 1 300 m to 2 100 m in Nanjian Co., Yunnan.

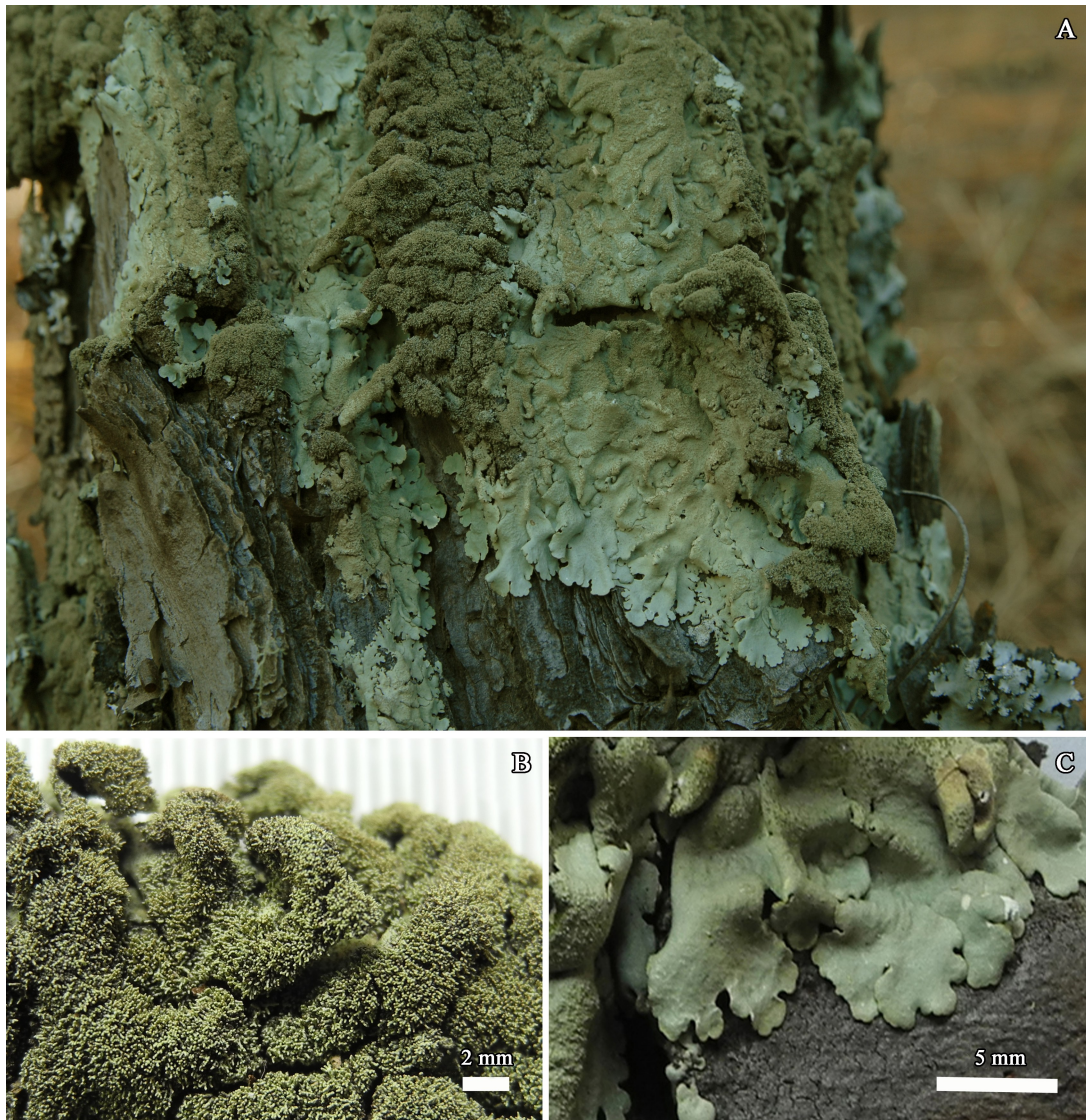


Fig. 2 *Canoparmelia ecaperata*. A. Habitat; B. Cylindrical laminal isidia. C. Narrow and sublinear lobes.
(All based on Li S. Wang 12-37823)

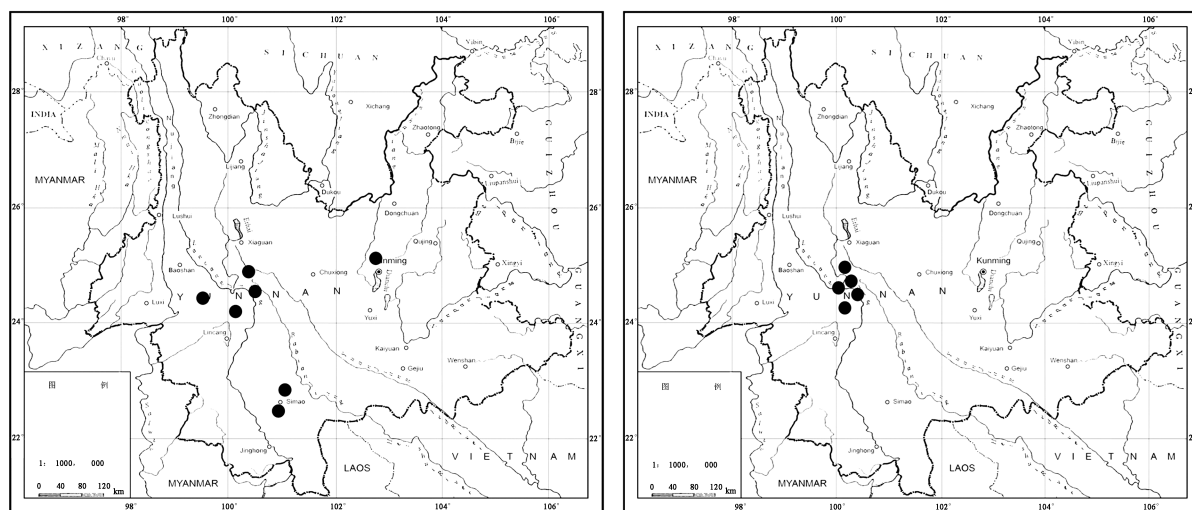


Fig. 3 Distribution map of *C. ecaperata* (left) and *C. texana* (right) from Hengduan Mountains

Remarks: *Canoparmelia ecaperata* is similar to *Parmotrema conformatum* and *Flavoparmelia caperata*. The former differs from *C. ecaperata* in the presence of fumarprotocetraric and protocetraric acids (P + red), ciliate lobes (Chen *et al.*, 2005; Benatti and Marcelli, 2009). Moreover, *C. ecaperata* occurs mainly in the tropical area of the Southern Hemisphere (Elix and McCarthy, 1998; Nöske and Sipman, 2004), whereas *C. ecaperata* has been reported from India and Thailand (Hale, 1976). *Flavoparmelia caperata* differs in the sorediate rather than isidiate lobes, and the presence of protocetraric acid rather than atranorin as the main compound. *Canoparmelia concrescens* might be confused with *C. ecaperata* but is distinguished by the bright yellow thallus (containing usnic acid in the cortex), and its distribution only in Africa. Finally *C. amazonica*, which also has isidia can be easily distinguished by the lack of usnic acid and spare rhizines.

Specimens examined: Yunnan Province: Nanjian Co.: Leqiu Village, N: 25°00'25.29", E: 100°21'43.50", alt. 2 060 m, on *Pinus yunnanensis* bark, Wang Lisong & Wang Xinyu 12-37814, 12-37820, 12-37822, 12-37823, 21 Dec. 2012; Gonglang Village, N: 24°43.103', E: 100°25.514', alt. 1 840 m, on *Pinus yunnanensis* bark, Wang Lisong *et al.* 12-33611, 25 March 2012. Longling Co., Songshan Mt., N: 24°44.672', E: 98°54.281', alt. 2 000 m,

on *Pinus*, Wang Lisong *et al.* 12-33440, 30 March 2012; Langcang Co., Mengla Village, on *Pinus*, alt. 1 100 m, Wang Lisong 10-31934, 11 Dec. 2010; Pu'er City, Ning'er Co., nearby road 213, N: 22°53'55.44", E: 101°02'28.41", alt. 1 334 m, on bark, Wang Lisong *et al.* 12-34474, 21 June 2012; Pu'er City, N: 22.48°, E: 100.58°, Zhao Ji-ding & Chen Yu-ben 2942, HMAS 100308, 18 Nov. 1960; Kunming City, Heilongtan, N: 25.04°, E: 102.42°, Zhao Ji-ding & Chen Yu-ben 4748, HMAS 100307, 16 Dec. 1960.

Canoparmelia texana (Tuck.) Elix & Hale in Mycotaxon **27**: 278, 1986. —*Parmelia texana* Tuckerman in American Journal of Science and Arts series **2** (253): 424, 1858. —*Pseudoparmelia texana* (Tuckerman) Hale in Phytologia **28**: 191, 1974.

Diagnostic characters: Thallus gray, upper surface covered with dense soralia, punctiform or capitate; lower surface black; moderately rhizinate, rhizines simple; containing atranorin and divaricatic acid.

Distribution: Pantemperate outside of Europe and pantropical at higher elevations. Shandong and Taiwan Province in China (Jia, 2008; Lai, 2000); New to Yunnan Province (Fig. 3).

Specimens examined: Yunnan Province, Nanjian Co.: Leqiu Village. N: 25°00'25.29", E: 100°21'43.50", alt. 2 060 m, on *Pinus yunnanensis* bark,

Wang Lisong & Wang Xinyu 12-37805, 12-37810, 12-37889, 21 Dec. 2012; Wuliang Medicine Valley, N: 24°52'2.76"; E: 100°34'51.39", alt. 2 345 m, on *Pinus amandii* bark, Wang Lisong & Wang Xinyu 12-37655, 19 Dec. 2012; Hongxing Village, Wuliang Medicine Valley, N: 24°50'40.41", E: 100°38'4.52", alt. 2 341 m, on bark, Wang Lisong & Wang Xinyu 12-36923, 16 Oct. 2012; Gonglang Country, Jinguo Village, N: 24°48.557', E: 100°24.839', alt. 2 270 m, on bark, Wang Lisong *et al.* 12-33742, 25 March 2012; Ziqiang Village, N: 24°46'10.55"; E: 100°25'08.13", alt. 1 640 m, on bark, Wang Lisong & Wang Xinyu 12-37674, 19 Dec. 2012; Ziqiang Village, N: 24°47'21.60"; E: 100°25'07.78", alt. 1 740 m, on bark, Wang Lisong & Wang Xinyu 12-37665, 19 Dec. 2012; Dadianshan Mt., N: 24°51'48", E: 100°34'39", alt. 2 543 m, on branch, Wang Lisong *et al.* 12-32957, 21 March 2012.

Canoparmelia amazonica (Nyl.) Elix & Hale in Mycotaxon **27**: 278, 1986

Thallus grey, upper surface covered with rare branched isidia, rhizines dense. Containing atranorin and protocetraric acid (Hale, 1976).

Canoparmelia crozalsiana (Bouly de Lesdain) Elix & Hale in Mycotaxon **27**: 278, 1986

Thallus greenish or gray, sorediate, moderately rhizinate. Containing atranorin, stictic acid, constictic acid (Hale, 1976).

Canoparmelia subtiliacea (Nyl.) Elix & Hale in Mycotaxon **27**: 279, 1986

Thallus grey, upper surface without soredia or isidia, rhizines rare. Containing atranorin and caperatic acid (Hale, 1976).

3 Discussion

Since the genera *Flavoparmelia*, *Parmotrema*, and *Canoparmelia* are similar in external morphology (Table 2) and therefore hard to distinguish from each other, the phylogenetic method was applied in this study to verify the systematic position of these taxa and validate the morphological identification. The genus *Parmotrema* is characterized by broad lobes (usually more than 5 mm) and rare, simple rhizines in the central part of the lower surface, upper surface grayish green, without usnic acid (Chen *et al.*, 2005); *Flavoparmelia* is characterized by broad and roundish lobes, containing usnic acid on the upper surface so that the color is yellow-green (Hale, 1986); *Canoparmelia* has sublinear and rather narrow lobes, closely attached to the substrate, has a subtropical to tropical distribution, and always contains atranorin as the main compound (Elix *et al.*, 1986).

Parmotrema conformatum was previously reported from Yunnan Province by Zhao *et al.* (1982), based, however, on two specimens (HMAS 100307 100308) that lacked cilia and had P- medullary reaction. Careful examination of the two specimens revealed that they belong to *C. ecaperata* according to morphological, chemical, and distributional characters. This is further supported by inferences from molecular data whereby both specimens belong to *Canoparmelia* and to *C. ecaperata* in particular.

Table 2 Key differences among *Canoparmelia*, *Flavoparmelia* and *Parmotrema*

	<i>Flavoparmelia</i>	<i>Parmotrema</i>	<i>Canoparmelia</i>
Thallus color	Yellowish-green	Grey to greenish	Grey to yellowish
Lobes	Broad (2-6 mm)	Rather broad (5-10 mm)	Medium (0.5-5 mm)
Lower surface	Black, with a narrow, brown, naked marginal zone	Brown to black, with broad naked zone.	Black, narrow, brown, marginal zone (<1 mm)
Rhizines	Sparse to moderate, simple or branched	Sparse and simple, in the thallus center	Moderate to dense, Simple or tufted
Characteristic compounds	Usnic acid	Atranorin	Atranorin, usnic acid
Distribution	Temperate	Temperate to tropical	Subtropical to tropical

The phylogenetic tree showed a final alignment consisting of 480 characters, of which 281 characters are constant, 31 variable parsimony-uninformative, and 168 parsimony-informative. All characters have equal weight. Gaps are treated as missing. The GC content of *C. ecaperata* is 54.7%, lower than *C. texana* (56.2%). The molecular data showed that the phylogenetic relationship of *C. ecaperata* is closer to *Canoparmelia*, which is in accord with our morphological identification. The final result confirms the placement of this species in the *Canoparmelia* clade. In this analysis, *C. ecaperata* is recovered as sister group of *C. texana* and *C. conrescens* with high support.

In conclusion, the presence of two taxa of *Canoparmelia* has been confirmed from the Hengduan Mountains based on morphological, chemical and phylogenetic study: *C. ecaperata* is reported as new to China and *C. texana* as new to Yunnan. The previous record of *Parmotrema conformatum* from China is based on misidentification (= *C. ecaperata*) which implies that the species should be deleted from the flora of China. The study added to the lichen biodiversity of the Hengduan Mountains, and also showed that there is still a high potential for new discoveries in this region.

Acknowledgment: We are grateful to Prof. Bernard Goffinet (University of Connecticut, USA) and Prof. Schmidt-Vogt Dietrich (KIB) for reviewing the manuscript, Ms. Deng Hong for helping with the original specimens study, Dr. Li Guodong, Dr. Yu Wenbin, Tang Kemin and Liang Mengmeng for their help with the molecular analysis, Dr. Ma Wenzhang for his assistance during our field work, Prof. Du Zhizhi and Zhang Hongxia for their support with the chemical analysis. We would also like to thank Yunnan Wei-he Pharmaceutical Co., Ltd. (云南维和药业股份有限公司) for the financial support.

References:

- Benatti MN, Marcelli MP, 2009. Species of *Parmotrema* (Parmeliaceae, Ascomycota) of the central-southern coast of São Paulo Estate, Brazil: I. Gyrophoric and lecanoric chemical group [J]. *Acta Botanica Brasiliica*, **23** (4): 1013—1026
- Chen JB, Wang SL, Elix JA, 2005. Parmeliaceae (Ascomycota) lichens in China's mainland III. The genus *Parmotrema* [J]. *Mycotaxon*, **91**: 93—114
- Culberson CF, Hör-Dur K, 1970. A standardized method for the identification of lichen products [J]. *Journal of Chromatography A*, **46**: 85—93
- Elix JA, Johnston J, Verdon D, 1986. *Canoparmelia*, *Paraparmelia* and *Relicinopsis*, three new genera in the Parmeliaceae (lichenized Ascomycotina) [J]. *Mycotaxon*, **27**: 271—282
- Elix JA, McCarthy PM, 1998. *Catalogue of the Lichens of the Smaller Pacific Islands* [M]. Bibliotheca Lichenologica, 70, J. Cramer, Stuttgart, 361
- Gardes M, Bruns T, 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts [J]. *Molecular Ecology*, **2** (2): 113—118
- Hale ME, 1976. A monograph of the lichen genus *Pseudoparmelia* Lynge (Parmeliaceae) [J]. *Smithsonian Contributions to Botany*, **31**: 1—62
- Hale ME, 1986. *Flavoparmelia*, a new genus in the lichen family Parmeliaceae (Ascomycotina) [J]. *Mycotaxon*, **25** (2): 603—605
- Hawksworth DL, Kirk PM, Cannon PF *et al.*, 1995. *Ainsworth & Bisby's Dictionary of the Fungi* [M], 8th ed. Wallingford: CAB International, 1—616
- Jia ZF, Li YL, Hou J *et al.*, 2008. A study of lichen genus *Canoparmelia* from Taishan Mountain [J]. *Mycosystema*, **27** (3): 461—463
- Lai MZ, 2000. *Illustrated Macrolichens of Taiwan I* [M]. Taipei: Council of Agriculture, 106—107
- Nöske NM, Sipman HJM, 2004. Cryptogams of the reserva biológica san francisco (Province Zamora-Chinchipe, Southern Ecuador) II. Lichens [J]. *Cryptogamie, Mycologie*, **25** (1): 91—100
- Orange A, James PW, White FJ *et al.*, 2001. *Microchemical Methods for the Identification of Lichens* [M]. London: British Lichen Society, 1—101
- Singh K, Sinha G, 2010. *Indian Lichens: an Annotated Checklist* [M]. India: Botanical Survey of India, 1—572
- Swinscow T, Douglas V, Krog H, 1988. *Macrolichens of East Africa* [M]. London: British Museum (Natural History), 1—383
- Thell A, Crespo A, Pradeep K *et al.*, 2012. A review of the lichen family Parmeliaceae-history, phylogeny and current taxonomy [J]. *Nordic Journal of Botany*, **30**: 641—664
- Wei JC, 1991. *An Enumeration of Lichens in China* [M]. Beijing: International Academic Publishers Ltd., 1—278
- White TJ, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics [A]. In: Innis MA, Gelfand DH, Sninsky JJ *et al.* (eds.), *PCR Protocols* [M]. San Diego: Academic Press, 315—322
- Wolseley Y, Patricia A, Aguirre-Hudson B *et al.*, 2002. Catalogue of the lichens of Thailand [J]. *Bulletin of the Natural History Museum: Botany*, **32** (01): 13—59
- Yoshimura I, 1974. *Lichen Flora of Japan in Color* [M]. Osaka: Hoikusha Publishing Co., Ltd., 124—159
- Zhao JD (赵继鼎), Xu LW (徐连旺), Sun ZM (孙曾美), 1982. *The Preliminary of Lichen in China* (中国地衣初编) [M]. Beijing: Science Press, 41—43